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VENTRICULAR DILATATION OVER SEVERAL WEEKS FOLLOWING INDUCTION OF EXCITOTOXIC (SYSTEMIC LITHIUM/PILOCARPINE) LESIONS: POTENTIAL ROLE OF DAMAGE TO THE SUBSTANTIA NIGRA RETICULATA

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Time-dependent atrophy of cerebral space and enlargement of the lateral ventricles were noted in healthy rats 1 to 100 days after the induction of seizures by a single systemic injection of lithium and pilocarpine. The rate of atrophy was most strongly correlated (0.90) with the log (base 10) of the time in days. Most of the degeneration had occurred within about 20 to 30 days after the seizure-induced brain trauma. Concomitant reduction in the area of the substantia nigra reticulata was the most powerful predictor of ventricular enlargement at the level of the caudate-putamen.

Keywords: Seizure; substantia nigra reticulata; thalamus lateral ventricles; rat; insidious necrosis

Ventricular dilatation subsequent to diffuse traumatic injury to the human brain has been considered a strong inference of parenchyma degeneration (Cullum and Bigler, 1986; John, Govindaraju, Raghunathan and Kumar, 1996). In a recent human case study for which a premorbid MRI (magnetic resonance imaging) was available (Bigler, Kurth, Blatter and Abildskov, 1992), enlargement of the lateral ventricles subsequent to a severe closed

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head injury had approached asymptote within about one month following the trauma. MRIs of the lateral ventricles following induction of a neurotoxic lesion also displayed time-dependent enlargement (John *et al.*, 1996). Identification of the specific temporal parameters that are associated with ventricular dilatation (particularly in conjunction with adjacent regional necrosis) may reveal principles from which strategic interventions could be developed.

We (Persinger, Makarec and Bradley, 1988; Persinger *et al.*, 1993; Persinger, Bureau and Peredery, 1994) have been investigating the temporal patterns of necrosis within rat brains in which seizures were induced by a single injection of lithium (3 mEq/kg, s.c.) that was followed 4 hr later by an injection of pilocarpine (30 mg/kg, s.c.). Within three days of the induction of the seizures (even though overt symptoms have terminated) some degree of neuronal necrosis or reactive gliosis can be clearly discerned by light microscopy (100 X and 400 X) within the cerebral cortices and within dozens of different discriminable nuclei within the thalamus and subcortical telencephalon. The only damage that is discernable at this level of magnification within the midbrain occurs within the substantia nigra reticulata (SNr) within the region that receives thalamic input.

The specific involvement of the SNr would be compatible with Gale's (1988) conclusion that this subcomponent of the tegmentum is the last major impediment before electrical seizures propagate into motor centers and become manifested as overt paroxysmal sequences. Damage to this area would be expected to allow the indiscriminate generalization of electrical seizures whose frequency or intensity could be so extreme that they would foster additional, insidious brain injury. Sustained electrical stimulation, through other pathways, has been shown to evoke highly reproducible patterns of damage within target regions (Sloviter, 1983).

Prediction that the amount of damage within the midline nuclei of the thalamus should affect the amount of transcerebral damage (and hence ventricular enlargement) was derived from the role of this portion of the thalamus in the regulation of both electrical seizures and arousal. Miller (1992) has concluded that the midline group facilitates the propagation of paroxysmal discharges throughout the brain (Miller, Gray and Bardgett, 1992). Because midline (and intralaminar) nuclei are primary sources of thalamostriatal fibers (Bentivoglio, Balceria and Kruger, 1991), anomalous stimulation from these areas would be expected to promote neuronal necrosis or atrophy within target regions. Such changes, considering the macrostructure of the rat brain, could be reflected by enlargement of the adjacent lateral ventricles.

METHOD

Subjects

Twenty (20), male Wistar albino rats (90 to 120 days of age) obtained from Charles River (Quebec) were injected (s.c.) with 3 mEq/kg of lithium chloride and then 4 hr later with 30 mg/kg of pilocarpine (Persinger, Makarec and Bradley, 1988; Persinger, Bureau, Kostakos, Peredery and Falter, 1993).

Procedure

To minimize mortality the rats were injected with 25 mg/kg (s.c.) of acepromazine (Atravet) approximately 30 min after the display of limbic motor seizures (rearing and forelimb clonus). The rats displayed the typical lateral hypothalamic-like syndrome and loss of body weight for five to seven days during which time they would only consume milk and bread or (Purina) rat chow in water (mush). After about 10 days the rats appeared normal except for brief (30 sec) overt limbic seizures (about 1/day) from which they immediately recovered as defined by the return to the previous activity, e.g., eating, grooming. All rats were treated in accordance with the C.C.A.C. (Canadian Council on Animal Care) guidelines.

Between 1 and 100 days after the induction of the seizure the rats were randomly selected and decapitated. Brains were removed, fixed in ethanol-formalin-acetic acid, processed and then embedded in paraffin (Persinger, 1980). Ten micrometer coronal sections (cut by microtome), selected from comparable regions between the level of the SNr (substantia nigra reticulata) and the CP (caudate putamen) were stained with toluidine blue O for each rat. Cross-sectional areas of the entire section and the lateral ventricles were obtained by projecting each slide onto 8 × 11 (1 mm) graph paper; verification was completed with a compensating polar planimeter (Sokkisha # 09438). The relative area (percent) of the lateral ventricle was obtained by dividing the cross-sectional area of both ventricles by the cross sectional area of the entire section for each of the two levels. The area of the reticulata portion of the substantia nigra was calculated in a similar manner by adding the values for the left and right sides of this structure over 3 to 4, 10 μm sections that were equally spaced longitudinally.

All of the structures, as defined by Paxinos and Watson (1986) were examined (two to four slides per structure) for the thalamus and the subcortical telencephalon [see Note 1]. The estimate of the total area of

damage, as defined by either conspicuous neuronal dropout or gliosis (400 *x*), along a 0 to 10 scale was completed for each structure by averaging (mean values) the results of the two to four slides per structure. For this study the structures were then organized according to traditional major regions [see Note 2]. Although our hypothesis emphasized the role of the midline group, the others were included as "controls" for generalized damage.

The first stage of the analysis was to determine the strength of the relationship between the ventricular enlargement and other inferences of neuronal damage and the time (in days) since the induction of the seizures. Because the relationship was clearly non-linear, both square root transformation and log (base 10) transformation of the time was computed. Pearson *r* and Spearman (rho) correlations were completed between the histological or volumetric measures and the temporal parameters.

To determine which neuronal groups contributed most to the enlargement of the ventricular areas, multiple regression analyses were completed between these measures and the extent of the damage within the selected brain regions and the SNr. Because of the limited sample size, the number of variables that could enter the step-wise regression was set at 3. Partial correlation analyses were completed between the relative areas of the ventricles, SNr, damage to the midline group and time in order to help clarify the direction of the shared sources of variance. Because of the size of the sample and our interest in major effects, the probability level for acceptable statistical significance was set at $p < 0.01$. All analyses involved SPSS software on a Vax 4000 computer.

RESULTS

There was a moderate strength correlation ($r = 0.84$, $p < 0.001$) between the two areas of the cerebrum. The changes in the cross sectional area of the cerebrum at the level of the caudate-putamen and the substantia nigra as a function of time after the inductions of the seizures were comparable. Sample cross sections of brains at the level of the caudate-putamen are shown in Figure 1 while the relationship between time and dilation at the level of the CP is shown in Figure 2. The correlations (*r*) between brain area and the log (base 10) of the days were −0.89 at the level of the CP and −0.86 at the level of the SNR. For both levels, the asymptote occurred within approximately 30 days and was associated with a terminal loss of about 20% to 25% of the normal cross-sectional area. For reference, the ranges of areas for the cerebrums from control rats who were of comparable age were

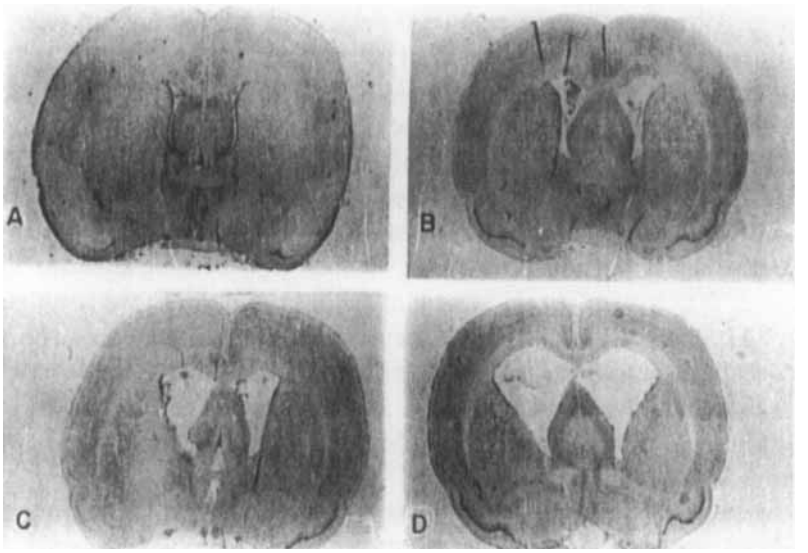

FIGURE 1 Sample brains at the level of the caudate putamen to demonstrate enlargement of the lateral ventricles.

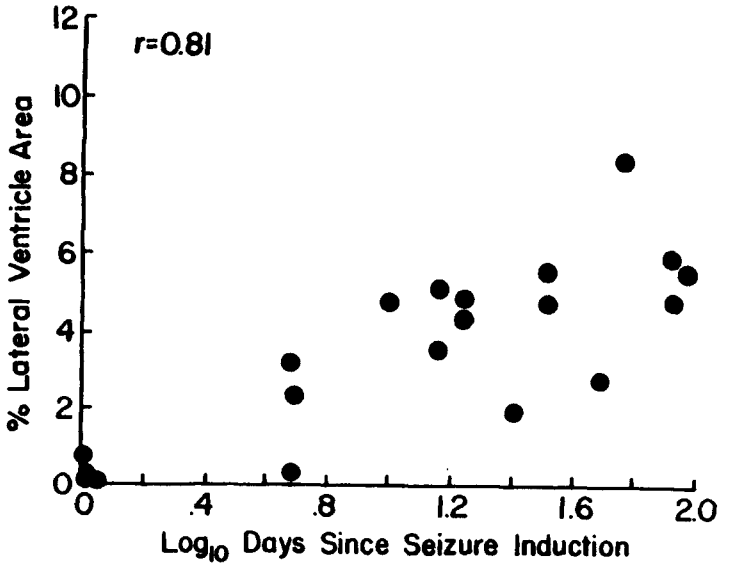

FIGURE 2 Percentage area occupied by the lateral ventricles relative to the total cerebral area at the level of the caudate putamen as a function of the $\log_{10}$ of days since the trauma.

between 350 mm-sq and 370 mm-sq at the level of the SNr and between 315 mm-sq and 330 mm-sq at the level of the CP.

To verify that the visually conspicuous enlargement of the ventricles was not an artifact, the percentage areas of the lateral ventricles were correlated with the log transformation of time since the brain trauma. The proportional area of the lateral ventricles at the level of the CP was strongly associated ($r = 0.81$) with the duration of time that had elapsed since the injury; a comparable but weaker ($r = 0.64$) relationship was observed for ventricular enlargement at the level of the SNr; the correlation ($r = 0.47$) between the relative ventricular areas for the two levels was not statistically significant ($p > 0.01$). The change in the cross-sectional area of the SNr over time is shown in Figure 3. The reduction was associated with conspicuous neuronal dropout and gliosis within the region of the SNr that receives thalamic input.

The means and standard deviations for the percentage of the area that exhibited neuronal damage or gliosis within the general thalamic and subcortical telencephalic regions are shown in Table I. The Pearson

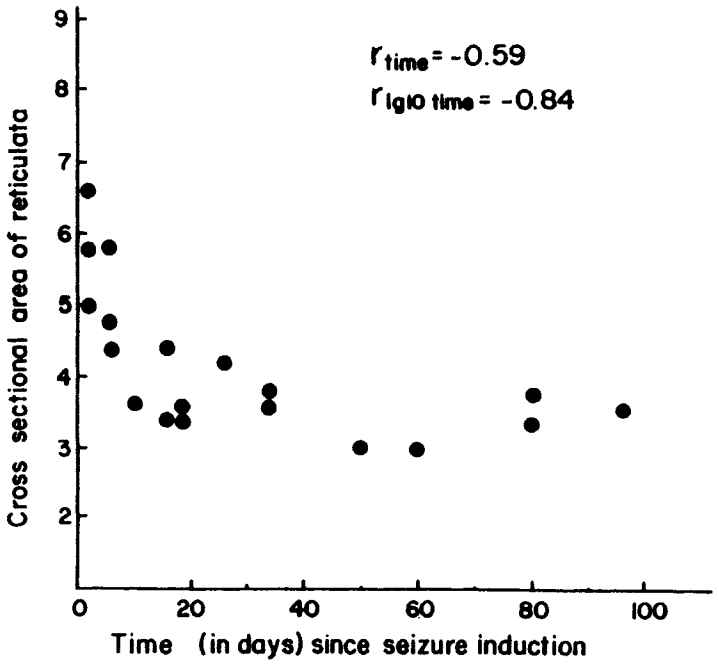

FIGURE 3 Mean area of the reticulata portion of the substantia nigra as a function of time since the brain trauma induced by lithium pilocarpine-evoked seizures.

TABLE I Means and standard deviations for proportional area of damage within thalamic and subcortical telencephalic regions, areas of the reticulata of the substantia nigra and lateral ventricles at the level of the caudate-putamen and SNr and the relative ventricular size. The correlations between these values and either real time (0 to 100 days) of the log (base 10) of the time since the seizure induction are also shown

Structure	*M*	*SD*	*Real Time*	*Log10 Time*
SN retic (mm)	4.2	1.0	−0.56	−0.83**
Ventricle CP (%)	3.9	2.7	0.65*	0.85**
Ventricle SNr (%)	3.0	1.7	0.63*	0.52
Cort. Amygdala (%)	39	22	0.08	−0.05
Lateral Amygdala (%)	32	21	−0.07	−0.24
Basal Amygdala (%)	26	19	−0.55	−0.63*
Midline Thalamus (%)	37	26	0.62*	0.54
Intralam Thalamus (%)	46	34	0.52	0.51
Mediodorsal Thal (%)	46	25	0.76**	0.66*
Lateral Thalamus (%)	60	28	0.87**	0.71**
Posterior Thalam (%)	45	31	0.91**	0.78**
Ventral Thalamus (%)	46	31	0.49	0.21
Metathalamus (%)	36	25	0.77**	0.69**
Hippocampal Form (%)	24	15	0.36	0.66*

*$p < 0.01$. **$p < 0.001$.

correlations between the development of damage within these areas and both the real time and log (base 10) of the time are included. For comparison the correlations between these two temporal references (square-root transformation of days since the brain trauma produced intermediate values) and the relative ventricular volumes are also shown. With the exception of the amygdaloid groups, the area of damage within the regions increased with time after the initiation of the seizures.

Multiple regression analyses ($F_{2,14} = 22.35$, $p < 0.001$; MR = 0.88) showed that only the cross sectional area of the SNr (beta = −0.67) and the percentage area of damage within the midline nuclei of the thalamus (beta = 0.33) entered the equation and were sufficient to explain 66% of the variance for the relative ventricular volume at the level of the caudate-putamen. The standardized partial regression coefficients indicated that as cross-sectional area of the reticulata decreased (an inference of neuronal loss) and the proportion of damage within the midline group increased, the relative size of the lateral ventricles at the level of the caudate-putamen also increased (see Fig. 4). There was no statistically significant ($p > 0.01$) correlation between the area of the SNr and the extent of damage within the midline group (−0.45). On the other hand, only the relative area of damage within the posterior group of the thalamus entered the equation for predicting the volume of the lateral ventricles at the level of the SNr. The

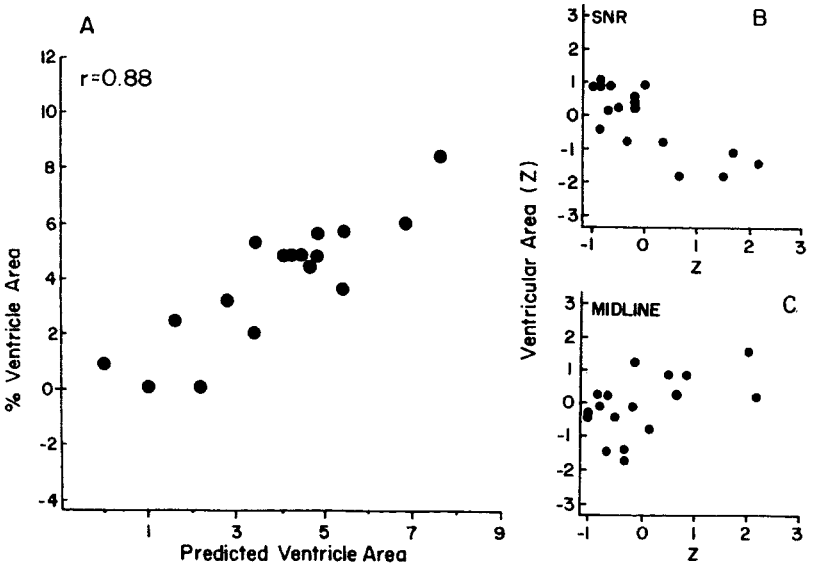

FIGURE 4 Scattergram of the relationship between the area of the reticulata of the substantia nigra and the area of damage within midline nuclei and the relative area of the lateral ventricles at the level of the caudate putamen. Partial plots for the contribution of each structure are also shown.

correlation (r) was 0.66 [$F_{1,15} = 11.82$, $p < 0.01$]. None of the other measures of damage within the subcortical telencephalic or diencephalic regions entered the equation for this dependent variable.

Partial correlation analyses verified that when the shared variance between the amount of damage within the midline thalamic group and the area of the SNr was first removed (held constant), the area of the SNr was significantly correlated with the ventricular size at the level of the caudate putamen (-0.78, $p < 0.001$) but not at the level of the SNr (-0.21). On the other hand, when the shared variance between the SNr and the midline group was first removed, marginally significant ($0.01 < p < 0.05$) associations remained between the midline group and the relative ventricular area at both the level of the caudate-putamen ($r = 0.52$) and the SNr (0.42).

When the shared variance between the midline thalamus and the SNr and the log(10) of time was first removed, the correlation between this temporal index and the relative area of the lateral ventricle at the level of the caudate-putamen was reduced to 0.44 ($p = 0.05$). Similarly, when the shared variance between these two subcortical variables and the log (10) of time was first removed, the strength of the association between the proportional size

of the lateral ventricle and the area of the reticulata (– 0.40) and the extent of damage within the midline thalamus (0.41) was no longer significant statistically.

DISCUSSION

Previous studies (Honchar, Olney and Sherman, 1983) have shown that the pattern and amount of neuronal necrosis within the various nuclei within the thalamus and the hippocampal-amygdaloid complex, about 24 hr after the initiation of the overt seizures by lithium (3 mEq/kg) and pilocarpine (30 mg/kg), are extremely similar to the pattern induced by a single larger dosage (380) of pilocarpine (Bureau, Peredery and Persinger, 1994). Animals that do not display the overt motor limbic seizures within 70 min following the injection of lithium/pilocarpine, never display discernable (light microscopy at 400 X) brain damage. Consequently, the neuronal damage is not due to the direct toxic effects of either the lithium or the pilocarpine.

Although enlargement of the lateral ventricles along two coronal planes was strongly time-dependent, the correlative structures that were associated with these changes differed. Only one thalamic group: the lateral group (of the multiple possible diencephalic and subcortical telencephalic groups that could have entered the equation) predicted the relative enlargement of the ventricles at the level of the SNr. Considering its adjacency to the actual ventricles, this observation could have been confounded by some spatial constriction. However such an artifact was not present for the enlargement of the lateral ventricles at the level of the caudate-putamen. The amount of damage within two structures, the SNr and the thalamic midline group, which have been associated with both modulation and propagation of electrical seizures (Iadarola and Gale, 1982) strongly predicted the proportional increase in the ventricular area. These results are consistent with the interpretation that the functional loss of these two "impediments" allows unmodulated neuroelectrical input to evoke necrosis or apoptosis within portions of the cerebral cortices and caudate-putamen.

If the SNr is the final gateway, as Gale (1988) predicts before the generalization of seizures into motor convulsions, then reduction of its size due to loss of (particularly GABA-nergic) neurons would facilitate their propagation (Iadarola and Gale, 1982). Correlative excitotoxic death within the corpus striatum, which is the major structure surrounding the ventrolateral portions of the anterior lateral ventricles, would enhance the expansion of these latter structures. The fact that the strong correlation

between the inference of damage within the SNr and the anterior ventricular area was still present even when the contribution of variance from the midline nuclei had been removed supports the powerful role of this structure upon imminent neuronal atrophy.

The estimated inflection for the relationship between ventricular enlargement and reduction of the cross-sectional area of the reticulata portion of the substantia nigra was about 20 days after the induction of the seizures and the ensuing brain damage. However anomalous processes within the brain may continue. We have also shown previously that an appearance of large aggregates of Nissl-staining debris and crystallized deposits of calcium embedded within a matrix of protein polysacharrides emerge after about 30 days (Lafreniere, Peredery and Persinger, 1992). These changes occur even though the rats appear to be "recovered" behaviourially.

We have been pursuing the possible relationships between traumatic brain injury, the initiation of subclinical partial complex (or limbic) seizures and the emergence of the schizophreniform syndromes (Andreasen *et al.*, 1994) in human contexts (Persinger, 1993, 1994). Although the conspicuous morphometric changes, as discerned by MRI or CT, may often approach an asymptote within one or two months in patients who have sustained traumatic brain injuries, the subsequent alterations in personality (Swerdlow and Koob, 1987) and its modification by subclinical complex partial epileptic lability (Ardila and Gomez, 1988; Roberts and Burton, 1990) may occur for years.

Presently about 20% to 30% of epileptic patients display a form of epilepsy that is difficult to treat by pharmacological agents, even when compliance by patients is maximum. About 80% of these drug resistant epilepsies involve electrical activity within the temporal cortices, amygdala, entorhinal cortex and hippocampus (Heinemann, Zhang and Eder, 1993). The life-time rates of spontaneous seizures, which are very similar to the brief (about 30 sec) displays associated with stage IV kindling, has been estimated to be about 1 per day. We suspect that subclinical electrical paroxysmal activity may be very persistent in these rats and: 1) may be a primary source of the insidious neuronal loss, and 2) may encourage "kindling"-induced synaptic reorganization.

If these assumptions are valid, then regular but intermittent application (across the temporal-limbic regions) of complex weak magnetic fields that persistently interfere with the recruitment of labile neurons into discharging aggregates (Bureau and Persinger, 1995) may be a method by which the insidious damage can be attenuated or stopped. Although polypharmacol-

ogy may be useful anticonvulsants for epileptic foci whose morphology is associated with marked alterations in receptor expression, these ligands still mediate their effects by synaptic mechanisms rather than directly affect the neuroelectrical and neuromagnetic fields which are generated by the aggregates of epileptogenic neurons. Weak, complex magnetic fields have the potential to modulate neural aggregates by directly affecting their emergent properties.

Notes

(1) They were (with the composite nuclei specified according Paxinos and Watson's nomenclature): the cortical amygdala [PMCO, PLCO, ACO, CXA], lateral amygdala [LADL, LAVL, LAVM], basal amygdala [BLA, BLP, BLV, BM], midline thalamus [PVA, REU, RHO, G, HAB], intralaminar thalamus [CLI, CM, PC, PF], lateral thalamus [LPMR, LPLR, LPMC, LPLC, LDDM, LDVL], posterior thalamus [PO, POT, ANG, SG, PLI], ventral thalamus [VM, VL, VPM, VPL, AVVL, GU], mediodorsal group [MD, IAM, MDM, MDC, MDL] of the thalamus, metathalamus [VLG, DLG, MG, MGD, MGV, MGM] and the hippocampal formation [CA2, CA2, CA3, CA4, DGY, SUB].

(2) The mean of the values for all of the structures within each region was obtained and multiplied by 100 to represent the percentage of its area that exhibited damage. Unlike the SNr, where necrosis was associated with a reduction in cross-sectional area due to the "collapse" of the structure, the thalamic regions appeared to maintain their general space. Consequently the estimates of neuronal drop out rather than cross sectional areas were employed.

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